Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Claims 1 and 2. (Previously Canceled)

3. (Previously Presented) The method of claim 40, wherein the retention layer comprises a particulate material.

4. (Previously Presented) The method of claim 3, wherein the particulate material of the retention layer consists of glass beads.

5. (Previously Presented) The method of claim 40, wherein the retention layer comprises a rigid retention material.

6. (Previously Presented) The method of claim 5, wherein the rigid retention material comprises sinter plates.

7. (Previously Presented) The method of claim 40, wherein the clarification reactor has a top and a bottom, wherein the retention layer is disposed inside the clarification reactor between the top and the bottom of the clarification reactor,

wherein the mixture comprising the precipitate and the lysate enters the top of the clarification reactor, and the lysate exits the bottom of the clarification reactor, with the precipitate being retained within the clarification reactor by the retention layer,

wherein in step d), increasing pressure is applied at the top of the clarification reactor to the mixture comprising the precipitate and the lysate, thereby ensuring a constant outflow of the lysate from the bottom of the clarification reactor.

8. (Currently Amended) The method of claim 48, wherein the applied pressurized gas is ~~pressure is increased by applying~~ pressurized air.

9. (Previously Presented) The method of claim 40, wherein one or more wash steps are inserted between steps d) and e).

10. (Previously Canceled)

11. (Previously Presented) The method of claim 40, wherein the flow of the cell suspension and the flow of the lysis solution are combined, without further mixing, before entering the lysis reactor, thus forming a single flow within the lysis reactor that is homogeneously mixed when flowing through the filling elements in the lysis reactor.

12. (Previously Presented) The method of claim 40, wherein the cell suspension and the lysis solution are introduced into the lysis reactor in the form of two independent flows.

13. (Currently Amended) The method of claim [[12]] 16, wherein ~~the two flows are introduced from independent sources connected through~~ the neutralization reactor includes a T-type or Y-type connector and a tubing system fluidly connecting the lysis reactor to the clarification reactor, the tubing system being in the form of a coil, wherein lysed cell solution and the neutralization solution are combined through the T-type or Y-type connector, thus forming a single flow through the tubing system, wherein the lysed cell solution and the neutralization solution are mixed to produce the mixture comprising the lysate and the precipitate during transportation through the tubing system between the lysis reactor and the clarification reactor, wherein the tubing system is configured to avoid shearing of flocks of the precipitate.

14. (Currently Amended) The method of claim 12 [[or 13]], wherein the two flows are transported at a defined ratio of flow rates, the flow rates being regulated by pressure or pumps, thereby ensuring a constant ratio of cell suspension and lysis solution volumes.

15. (Previously Presented) The method of claim 40, wherein in step c), the lysed cell solution obtained in step b) is mixed with the neutralization solution in a continuous mode.

16. (Previously Presented) The method of claim 15, wherein the lysed cell solution and the neutralization solution are combined at a constant ratio of flow rates.

17. (Previously Presented) The method of claim 40, wherein a concentration and/or a conditioning step is inserted between step d) and step e).

18. (Currently Amended) The method of claim 17, wherein [[a]] the concentration step and [[a]] the condition step are inserted, and wherein said concentration step takes place before said conditioning step.

19. (Previously Presented) The method of claim 40, wherein said biomolecule of interest is a polynucleotide.

20. (Previously Presented) The method of claim 19, wherein the polynucleotide is plasmid DNA.

Claims 21 and 22. (Previously Canceled)

23. (Previously Presented) The method of claim 40 wherein, in addition, step a) is operated in a continuous mode.

24. (Previously Presented) The method of claim 40, wherein the cell suspension ~~obtained~~ provided in step a) is obtained from a cryo-pelleted cell suspension.

Claims 25 – 39 (Canceled)

40. (Currently Amended) A method of purifying a biomolecule of interest from [[a]] host cells [[cell]] using an automated or semi-automated device, wherein the device comprises a lysis reactor, a ~~neutralizing~~ neutralization reactor and a clarification reactor fluidly connected to one another, the method comprising:

a) providing a cell suspension of host cells that have been cultivated to produce [[the]] a biomolecule of interest, wherein the cell suspension is a fermentation broth within which the host cells were cultivated or a re-suspension of the cultivated host cells that were harvested from the fermentation broth;

b) introducing a flow of the cell suspension and a flow of a lysis solution into the lysis reactor into the lysis reactor, the lysis reactor containing ~~wherein the lysis reactor contains~~ filling elements made of glass, plastic, stainless steel or fibrous material, ~~wherein~~ such that the flow of the cell suspension and the flow of the lysis solution into and through the lysis reactor provides homogenous mixing of the flows in the absence of shear forces and whereby the cultivated host cells are substantially disintegrated by alkaline lysis to produce a lysed cell solution ~~are homogenously mixed as a result of flowing through the filling elements in the lysis reactor so that irreversible denaturation of the biomolecule of interest is avoided and the cultivated host cells are disintegrated by alkaline lysis in the absence of shear forces in the lysis reactor to produce a lysed cell solution~~;

c) ~~transporting~~ neutralizing the lysed cell solution via the neutralization reactor wherein the lysed cell solution is mixed with a neutralization solution to produce a mixture comprising a lysate and a precipitate comprising cellular debris and impurities, and wherein the lysate contains the biomolecule of interest;

d) introducing the mixture comprising the precipitate and the lysate into the clarification reactor wherein the lysate containing the biomolecule of interest is separated from the precipitate, wherein the mixture comprising the precipitate and the lysate is allowed to flow through the clarification reactor, and wherein the

clarification reactor contains a retention layer that functions to retain the precipitate but allow the lysate to flow from the clarification reactor; and

e) purifying the biomolecule of interest, where the biomolecule of interest is purified from the lysate that flows from the clarification reactor,

wherein said method is operated on a manufacturing scale.

41. (Previously Presented) The method of claim 40, wherein one or more distribution means are disposed inside the clarification reactor and extend to a surface of the retention layer, wherein the one or more distribution means evenly distribute the mixture comprising the precipitate and the lysate as obtained in step c) into the clarification reactor of step d).

42. (Previously Presented) The method of claim 40, wherein the filling elements are a particulate material.

43. (Previously Presented) The method of claim 7, wherein the retention layer has a top facing the top of the clarification reactor and a bottom facing the bottom of the clarification reactor, wherein the retention layer functions to retain the precipitate on the top of and within the retention material while allowing the purified lysate to flow from the clarification reactor.

44. (Previously Presented) The method of claim 42, wherein the particulate material consists of beads, each bead having a diameter in the range of about 1 to about 100 mm.

45. (Previously Presented) The method of claim 44, wherein each bead has the same diameter.

46. (Previously Presented) The method of claim 42, wherein the lysis reactor is essentially completely filled with the particulate material.

47. (Previously Presented) The method of claim 42, wherein in the particulate material consists of glass beads.

48. (Previously Presented) The method of claim 7, wherein pressure is increased by applying pressurized gas.

49. (New) The method of claim 40, wherein the method is operated to process more than 100 grams wet cells and yield from 0.1 g to a kilogram of the biomolecule of interest.

50. (New) A method of continuously purifying a biomolecule of interest from host cells, the method comprising:

providing a device comprising a lysis reactor, a neutralization reactor and a clarification reactor, wherein the neutralization reactor fluidly connects the lysis reactor with the neutralization reactor, wherein the lysis reactor contains filling elements made of glass, plastic, stainless steel or fibrous material;

providing a cell suspension of host cells and a lysis solution, wherein the cell suspension of host cells have been cultivated to produce a biomolecule of interest, wherein the cell suspension is a fermentation broth within which the host cells were cultivated or a re-suspension of the cultivated host cells that were harvested from the fermentation broth;

simultaneously flowing the cell suspension and the lysis solution through the lysis reactor such that the cell suspension and the lysis solution homogenously mix when flowing through the filling elements in the lysis reactor without causing shear forces to the biomolecule of interest, wherein the cultivated host cells are substantially disintegrated by alkaline lysis in the lysis reactor to produce a lysed cell solution;

simultaneously flowing the lysed cell solution through the neutralization reactor with a neutralization solution, wherein the lysed cell solution is mixed with the neutralization solution to produce a mixture comprising a lysate and a precipitate comprising cellular debris and impurities, and wherein the lysate contains the biomolecule of interest;

flowing the mixture comprising the precipitate and the lysate through the clarification reactor, wherein the lysate containing the biomolecule of interest is separated from the precipitate, and wherein the clarification reactor contains a retention layer that functions to retain the precipitate but allow the lysate to flow from the clarification reactor; and

purifying the biomolecule of interest, where the biomolecule of interest is purified from the lysate that flows from the clarification reactor,

wherein said method is continuously operated on a manufacturing scale.